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SlowFade® Antifade Kits

S-2828 *SlowFade*® Antifade Kit
S-24635 *SlowFade*® Antifade Kit with DAPI
S-7461 *SlowFade*® *Light* Antifade Kit
S-24636 *SlowFade*® *Light* Antifade Kit with DAPI

Quick Facts

Storage upon receipt:

- Room temperature
- Protect from light (S-24635, S-24636)

Introduction

When exposed to excitation light, all fluorescent dyes fade (photobleach). The photobleaching is dependent on: 1) the intensity; and 2) the duration of illumination. The photon output of a dye represents the average number of cycles of excitation followed by fluorescence emission that the dye goes through before it is irreversibly photobleached. The average photon output is defined by the ratio of the probability that the dye will fluoresce (fluorescence quantum efficiency or Q_f) and the probability that it will photoreact irreversibly to become a nonfluorescent species (photobleaching quantum efficiency or Q_b). For example, fluorescein, which is very photolabile, has a Q_f/Q_b of about 30,000 in alkaline solution. Both Q_f and Q_b are properties of the dye that may be affected significantly by the dye's environment. The primary environmental influence on Q_b is the presence of singlet oxygen and free radical species. The main purpose of any antifade reagent is to sustain dye fluorescence. This is usually accomplished by inhibiting the generation and diffusion of reactive oxygen species, thereby reducing Q_b (preferably without any accompanying decrease in Q_f so that fluorescence will persist).

The active ingredient in Molecular Probes' *SlowFade*® Antifade Kits is 1,4-diazabicyclo[2.2.2]octane (DABCO), which appears to act as a free radical scavenger that extends useful fluorescence emission. Our original *SlowFade* formulation (S-2828) was designed to reduce the fading rate of fluorescein to almost zero (Figure 1); it does, however, decrease fluorescein's fluorescence intensity. Because it provides a nearly constant emission intensity, the original *SlowFade* is especially useful for quantitative measurements, as well as applications that employ extreme, prolonged or repeated excitation intensities such as confocal microscopy. This *SlowFade* formulation can extend the useful life of fluorescein's fluorescence by more than 50-fold, and can preserve the signal in cell and tissue mounts for up to two years (Dr. Robert Bacallao, Northwestern University, personal communication). The practical limits will depend significantly on the particular dye and its surrounding environment. We must caution that the original *SlowFade* formulation appears to excessively quench the fluorescence of 7-amino-4-methylcoumarin-3-acetic acid (AMCA) and Cascade Blue® fluorophores. We have also found that it is not generally effective as an antifade reagent for phycobiliproteins.

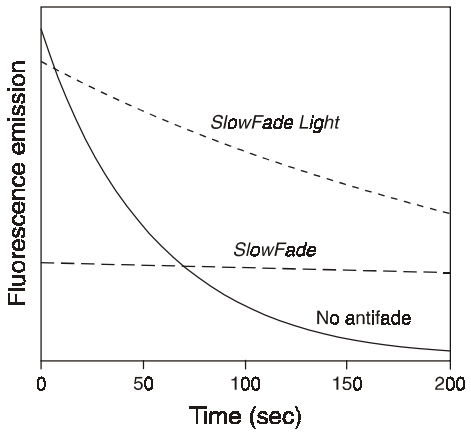

Figure 1. *The fluorescence intensity of fluorescein as a function of illumination time, under the following conditions: in the presence of the SlowFade Light antifade reagent ("SlowFade Light"), in the presence of the SlowFade antifade reagent ("SlowFade") and in the absence of an antifade reagent ("no antifade"). In these experiments, we added free fluorescein directly to a solution, and then examined the mixture in a capillary tube using a 20H/0.4 lens. In real samples such as cells and tissues, we find that the local environment influences the bleaching rates, yielding results that are sometimes qualitatively different from those shown here.*

The *SlowFade Light* Antifade Kit (S-7461) was developed to extend the useful fluorescence emission of fluorescein without significantly reducing fluorescein's initial fluorescence intensity. *SlowFade Light* slows fluorescein's fading rate by about five-fold with little quenching of fluorescein's initial fluorescence (Figure 1). This formulation is optimal for applications such as photomicroscopy where brightness and a good signal-to-noise ratio are required. Moreover, unlike the original *SlowFade* formulation, which quenches the fluorescence of AMCA and Cascade Blue dyes, *SlowFade Light* only minimally quenches the fluorescence of these blue fluorophores. We have found that *SlowFade Light* reduces the fading rate of the Cascade Blue fluorophore to almost zero, while decreasing its emission intensity by only about 30%.

Our experiments show that both *SlowFade* formulations provide better protection against fading when used at an appropriate pH. To maximize the performance of these products, we include a buffered wash solution with each kit.

For convenience, we also offer *SlowFade* and *SlowFade Light* formulation that include the popular nuclear counterstain DAPI. The bright blue fluorescence of this dye stands out in vivid contrast to green, yellow or red fluorescent probes of other structures, and its inclusion in the mounting medium eliminates the need for a separate counterstaining step. In most applications, DAPI stains nuclei specifically, with little or no cytoplasmic labeling. The absorption maximum for DAPI bound to dsDNA is 358 nm; the emission maximum is 461 nm.

Materials

Contents: Slowfade Antifade Kits (S-2828, S-24635)

- ***SlowFade* antifade reagent** (Component A), 10 mL in 50% glycerol (v/v) ready-to-use and sufficient for at least 200 coverslip-size experiments
- **2X concentrated *SlowFade* antifade reagent** (Component B), 2.5 mL provided for those applications in which glycerol may be incompatible
- **Equilibration Buffer** (Component C), 60 mL

Components A and B of kit S-24635 also contain DAPI at 0.2 µg/mL and 0.4 µg/mL, respectively.

Contents: SlowFade Light Antifade Kit (S-7461, S-24636)

- ***SlowFade Light* antifade reagent** (Component A), 10 mL in 50% glycerol (v/v) ready-to-use and sufficient for at least 200 coverslip-size experiments
- **2X concentrated *SlowFade Light* antifade reagent** (Component B), 2.5 mL provided for those applications in which glycerol may be incompatible
- **Equilibration Buffer** (Component C), 60 mL

Components A and B of kit S-24636 also contain DAPI at 0.2 µg/mL and 0.4 µg/mL, respectively.

Storage and Handling

When stored at room temperature, these solutions are stable for at least one year. They do not require refrigeration. The *SlowFade* and *SlowFade Light* Antifade Kits with DAPI should be stored protected from light.

Application

The *SlowFade* and *SlowFade Light* Antifade Kits are for non-living specimens only, including fixed cells, tissues and cell-free preparations. A representative specimen should be tested for compatibility with the antifade reagent prior to research applications. Careful fixation is important for maintaining the sample and staining integrity. The effects of *SlowFade* antifade reagent on binding affinities of dyes and ligands is currently undetermined.

These products are provided in two different solutions to allow their use in applications in which glycerol may be incompatible, such as mounting specimens containing lipophilic plasma membrane stains like DiI. The glycerol-containing solution is ready-to-use and recommended for most applications. The 2X concentrated solution can be diluted 1:1 in filtered distilled water to obtain a 1X glycerol-free solution. If glycerol is acceptable for the application, simply dilute the 2X concentrated solution with an equal volume of glycerol to generate additional glycerol-containing solution.

We have found that both *SlowFade* formulations provide much better protection against fading when used at basic pH. Prior to adding *SlowFade* or *SlowFade Light* antifade reagents, pre-equilibrate the specimen for five or ten minutes in the *SlowFade* Equilibration Buffer provided. To speed the equilibration process, the researcher may wish to rinse the specimen three or four times in the Equilibration Buffer. However, please use the Equilibration Buffer sparingly; we have supplied only enough for a few rinses. Remove as much Equilibration Buffer as possible before adding *SlowFade* or *SlowFade Light* reagents to the sample so as not to dilute the antifade reagent. If you choose not to employ the Equilibration Buffer, the sample should be almost dry before applying the antifade reagent. After applying one drop of antifade reagent, mount and seal the slide. Sealed samples may turn yellow within about a month of preparation, even when stored in the refrigerator. This color does not appear to affect the fluorescence or morphology of the specimen.

To further reduce photobleaching, minimize the exposure of fluorescently labeled specimens to light with neutral density filters and expose samples only when observing or recording a signal. Maximize collection of fluorescence by using a minimum of optics, high-numerical aperture objectives, relatively low magnification, high-quality optical filters and high-speed film or high-efficiency detectors.

DAPI exhibits absorption and emission maxima at 358 and 461 nm, respectively, and can be excited with a xenon or mercury-arc lamp, or with a UV laser. Suitable bandpass filter sets for viewing DAPI include filter sets XF06 and XF136 from Omega Optical Inc., or filter sets 31000 and 31013 from Chroma Technology Corp.

General References

- "Guiding principles of specimen preservation for confocal fluorescence microscopy," R. Bacallao *et al.*, in *Handbook of Biological Confocal Microscopy*, J. Pawley, Ed., pp 197–205, Plenum Press, New York (1990).
- "Photometric analysis of antifading reagents for immunofluorescence with laser and conventional illumination sources," G. Bock *et al.*, J Histochem Cytochem 33, 699 (1985).
- "1,4-Diazobizyklo[2.2.2]oktan (DABCO) verzogest das Ausbleichen von immunofluoreszenzpraparaten," G. Langanger, J. De Mey and H. Adam, Mikroskopie 40, 237 (1983).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
S-2828	*SlowFade*® Antifade Kit	1 kit
S-24635	*SlowFade*® Antifade Kit with DAPI	1 kit
S-7461	*SlowFade*® *Light* Antifade Kit	1 kit
S-24636	*SlowFade*® *Light* Antifade Kit with DAPI	1 kit

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